No. II.

(NEW SERIES.)

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

ON A PARASITE FOUND IN PERSONS SUFFERING FROM ENLARGEMENT OF THE SPLEEN IN INDIA, SECOND REPORT.

BY

LIEUT. S. R. CHRISTOPHERS, M.B., I.M.S.
(On special duty.)

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On a Parasite found in Persons suffering from Enlargement of the Spleen in India, Second Report.

In my first report I recorded briefly the result of the researches of different observers on the parasite recently discovered by Leishman and Donovan in cases of tropical splenomegaly. Such investigations, in many cases of enlarged spleen, have shewn in blood drawn during life from the spleen and liver or in films of these organs *post-mortem* the presence of certain very definite bodies of peculiar nature and uniform morphology. In the case of preparations made during life a matrix-like substance is conspicuous in which many of the bodies lie embedded. The actual nature of the matrix has given rise to much discussion. It is considered by Laveran and Mesnil to be altered red corpuscle, by Ross a parent mass producing the bodies, by Manson and Low it is termed a "zooglea mass." In my last report I gave many reasons for considering the matrix to be largely, if not entirely, the fragmented, budded and vacuolated cytoplasm of cells in which, in sections, the bodies are seen lying.

In all the researches mentioned the attention of the observer had been entirely occupied with the presence of the parasite in the blood.

Wright's announcement that similar or very closely related bodies occurred in large numbers in the tissues of tropical ulcer gave rise therefore to considerable surprise.

The microscopical appearances of tropical ulcer were described by Cunningham in 1885.[1] He notes extensive infiltration of the corium and subcutaneous tissues by granulation tissue containing bodies which he considered to be parasitic in nature. From Cunningham's drawings it would appear that these were macrophages or other cells containing the bodies described by Wright, though Cunningham figures no structure in these latter and terms them nucleoid bodies.

Wright[2] defines tropical ulcer as a single or multiple focal lesion of the skin characterised by the formation of elevated and indurated areas which ulcerate and eventually cicatrise. He draws attention to the resemblance to certain forms of cutaneous tuberculosis and syphilis. He describes, and gives photographs, shewing an extensive infiltration of the corium and subcutaneous tissue with cells. In addition to plasma cells and various kinds of lymphoid cells, he notes large cells with vesicular nuclei, the cytoplasm of which contains numerous peculiar bodies having a very constant morphology and structure. An examination of the excellent photographs of these bodies leaves no doubt as to their

morphological identity with the forms seen in cases of enlarged spleen in Madras. Wright describes the bodies as largely occurring in the cytoplasm of cells, especially of the large cells with vesicular nuclei. His photographs shew that the great majority of the bodies are situated in the cytoplasm of these cells which appear identical with those I have already described in the spleen, liver, and bone-marrow and which I have termed macrophages.

Wright's discovery would appear to modify considerably existing views as to the nature of the parasite, since the presence of the bodies in immense numbers in the cytoplasm of infiltrating cells in a focal skin lesion seems opposed to their supposed rôle as parasites of the red cell or even as solely parasites of the blood. Laveran and Mesnil,[3] however, still maintain the relation of the parasite to piroplasma. Examining slides sent to them by Donovan, they describe very small and rare forms in the peripheral circulation which may be free or may be situated in an unaltered red cell. The small size, the evident great rarity and especially the absence of the characteristic double chromatin masses make the relation of these forms to the parasite very doubtful. I have examined both peripheral and splenic blood for appearances which would seem to point to the origin of the bodies in the red cells, but without result.

In my first paper I drew attention to certain very definite clinical features of this disease which is, as a rule, readily diagnosed. I also described three autopsies and gave the result of an examination of some of the organs and tissues. In the present report I shall consider the results arrived at under the following heads.

(1) A description of four further autopsies.
(2) The salient and pathological nature of the disease.
(3) The relation of the parasite to the tissues of the host, especially demonstrating a type of infection in which the vascular endothelium is principally implicated.
(4) Certain points in the morphology of the parasite and in the nature of the "matrix" or "zooglea mass."
(5) A comparison of the conditions found *post-mortem* in infection with the new parasite with those found in trypanosomiasis.

Autopsies in fatal cases of the disease.

AUTOPSY 4.—Child aged about 12. Emaciation marked. Death from *cancrum oris.*

Spleen much enlarged, of firm consistence and dark red in colour, but not pigmented.

Liver considerably enlarged; very pale and mottled in appearance. On section the mottling was seen to be due to the presence of new tissue of pale hue replacing the centre of the lobule and extending about half way to the periphery.

The line of demarcation between the pale tissue and the darker liver tissue was very distinct. Microscopical examination shewed that the above appearances were due to the fact that in the centre of the lobule the liver cells were markedly atrophied, and the liver substance almost replaced by the large cells described in the last report.

Intestines.—The mucous membrane was pale, but otherwise quite normal in appearance.

The lymphatic glands behind the mesentery were about the size of beans and of normal appearance.

A large hæmorrhage was present under the peritoneum of the under surface of the diaphragm extending over several square inches. Several smaller hæmorrhages were present in the neighbourhood. Prior to death the spleen had not been punctured.

The œsophagus was examined closely with a lens but no unusual appearances were seen in the muscular coats.

The muscles were examined in several situations with a lens but shewed no unusual appearances.

The heart, kidneys, pancreas, suprarenals and *bladder* were normal in appearance.

The lungs shewed areas of congestion and there were small subpleural hæmorrhages over the bases.

Distribution of the bodies.—Bodies were present in large numbers in the spleen, liver and bone-marrow. Sections of the liver and spleen shewed, as in the tissues of the first three autopsies, many large cells crowded with the bodies lying in the capillaries. In the spleen large branched cells which appeared to assist in forming the splenic reticulum also contained many bodies in their cytoplasm. In blood from the hepatic veins bodies were seen in cells of mononuclear and endothelial type as well as in polymorphonuclear cells. In blood taken from a small vessel in the muscles of the thigh a fair number of bodies were present in large mononuclear cells.

Various connective tissues were closely examined for the parasites but without result.

Muscle spread upon the slide by the " half-drying method " and stained by the modification of Romanowski described in the first report did not reveal any bodies.

AUTOPSY 5.—Man aged about 40. Fairly well nourished. Death from peritonitis.

Abdomen contained about two pints of purulent fluid which was collecte especially in the neighbourhood of the cæcum.

Spleen somewhat enlarged. There were two large infarcts and many small ones visible.

Liver.—Nearly one half of the liver was light yellow in colour and necrotic in appearance. The condition appeared to be an enormous infarct. The remaining liver substance shewed, to a less degree, the lobular changes described in case 4. An abscess cavity containing necrotic tissue and about the size of a small orange was situated in the non-infarcted liver tissue.

Small intestine normal in the greater portion of its length. Peyer's patches in the lower few feet were prominent.

Large intestine except the cæcum shewed no marked changes. The cæcum over an area of several square inches was gangrenous and perforation at one point had taken place. The appendix was normal.

Stomach.—There were several extensive areas over which the mucous membrane was infiltrated with blood, and of a gelatinous consistence. Small areas of congestion were present in the neighbourhood of these areas.

Œsophagus.—The lower two inches of the œsophagus was occupied by a large sloughing ulcer which had completely destroyed the mucous membrane.

Kidneys were pale and shewed fatty degeneration of the convoluted tubules.

Pancreas was soft and flabby.

Heart.—The valves were normal. The endocardium was stained red.

Lungs.—There was marked congestion of both bases.

Brain.—Normal.

Skin.—On the skin of the front of the right thigh there was a mark resembling the cicatrix of a healed ulcer. On incision a deeply situated caseous mass, the size of a large pea, was disclosed surrounded by red granulation tissue and dense fibrosis. The skin over the caseous mass was thin and on the point of absorption, the appearance of a cicatrix being deceptive.

On the skin of the thighs numerous small pale areas could be detected apparently of the nature of cicatrices after superficial ulceration.

Distribution of the bodies.—Large numbers of the bodies were present in the spleen, liver and bone-marrow. Many single bodies were seen lying in cells in the granulation tissue around the caseous nodule in the skin. In the small vessels in the immediate neighbourhood of this tissue many large cells crowded with the parasites were seen. Sections of the normal skin of the thigh shewed no bodies, nor were the characteristic large cells seen in the vessels. A lymphatic gland in the right groin shewed numerous bodies in large cells in the lymph sinuses and in the stroma cells of the lymphoid tissue. In certain areas of the testis many single bodies were seen and large cells containing many bodies were seen in some of the small vessels. A few cells containing a number of bodies were found in films from the kidney substance and from the lung. Sections of the kidney did not reveal any parasites. In blood from the femoral vein bodies were found included in leucocytes and endothelial cells. In the films of different organs and in the blood many large

encapsuled bacilli were seen in leucocytes. In the capillaries of the brain some of the endothelium cells were packed with this organism. Some of these *post-mortem* appearances would appear to have been due to a secondary bacterial infection.

AUTOPSY 6.—Man aged about 45. Cause of death, large meningeal hæmorrhage.

Spleen large, firm, dark red, and not pigmented.

Liver large, pale and mottled. The lobules shewed the changes described in case 4.

Small intestine.—Normal.

Large intestine.—Numerous small ulcers extending to the muscular coats were scattered over the whole length of the gut. There were several raised plaques formed by papillomatous-like outgrowths. On microscopical examination the projecting mass was seen to be granulation tissue, and the crypts of Lieberkuhn in the neighbourhood were infiltrated with new tissue and in process of destruction.

Brain.—Under the dura mater of the left hemisphere there was a large clot covering the greater part of the cortex and occupying the space at the base of the brain. There was no fracture and no extravasation of blood outside the dura mater nor in the tissues of the scalp. There was no blood in the ventricles. The brain substance appeared normal.

The larger arteries were free from atheroma.

The heart, lungs, pancreas and *kidneys* appeared normal.

The skin.—There were no skin lesions.

The distribution of the parasite.—Bodies were present in large numbers in the spleen, liver and bone-marrow. They were not detected in an inguinal lymphatic gland nor in the skin. The distribution of the bodies in the other organs has not yet been worked out.

AUTOPSY 7.—Girl aged about 16. In hospital for large sloughing ulcer of the foot. Cause of death—*noma* of the vulva.

Spleen large, firm, dark, and not pigmented.

Liver large, pale and mottled. Lobular changes as in case 4, very marked.

Small intestine.—Normal.

Large intestine.—There were many depressed and pigmented areas scattered over the whole of the mucous membrane of the large bowel. Except that the ulcers had healed, the condition was very similar to that found in autopsy 6.

Skin.—On the inner side of the left heel was an ulcer about the size of a rupee. On the dorsum of the right foot was an ulcer the size of the palm of the hand, exposing the deep tissues and bones. The vulva was in a sloughy and very foul condition.

Distribution of the parasite.—Bodies were very abundant in the liver and spleen. The bone-marrow was not examined. In both ulcers single bodies were seen lying in the organised granulation tissue in the bases and also in the healthy tissues beyond. Large cells crowded with the bodies were seen here and there in the small vessels in the neighbourhood of the ulcers.

GENERAL *POST-MORTEM* APPEARANCES.—It will be seen from the seven autopsies now described that there are certain constant pathological appearances associated with infection by the new parasite. We may summarise these as follows.

The *post-mortem* appearances of the liver and spleen are in themselves almost pathognomonic. The spleen has a peculiar smooth, firm and solid look, and it retains its shape after removal from the body like an organ hardened *in situ*. The substance is dark red, granular and homogeneous. The trabeculæ shew up clearly against the dark pulp. The consistence is firm but friable and the sensation given to the finger is quite different to that given by a tough fibroid spleen. The liver retains its shape after removal. The liver substance is firm but friable. The surface is mottled, suggesting the nutmeg liver of passive engorgement, but the general colour is lighter instead of darker than normal. On section, the mottled look is seen to be due to a white tissue which occupies the centre of the lobules and gives rise to an arborescent appearance. Microscopical examination shews that the white tissue is a new deposit formed of macrophages and their contained bodies, which have practically replaced the liver tissue of the centre of the lobule.

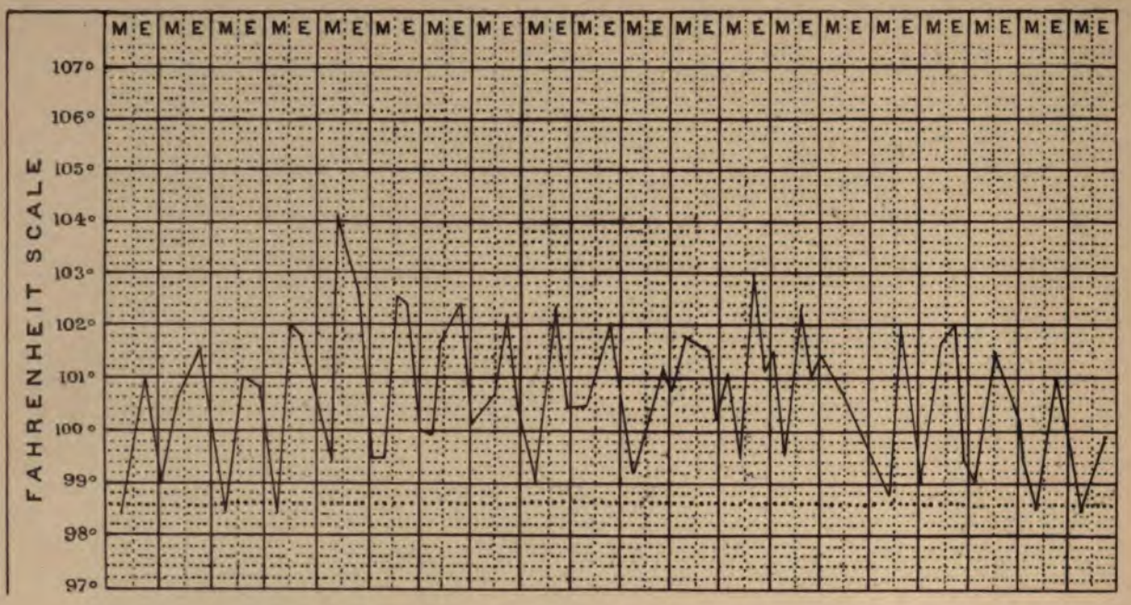
The large intestine shews, almost constantly, extensive multiple ulcers which are deep and sloughy and tend to perforate the muscular coats. The occurrence of fungating granulation tissue which has a papillomatous appearance is seen in association with the ulcers.

Purulent peritonitis and bronchopneumonia are frequent, arising, respectively, from the perforation of intestinal ulcers and as a sequel to *cancrum oris*.

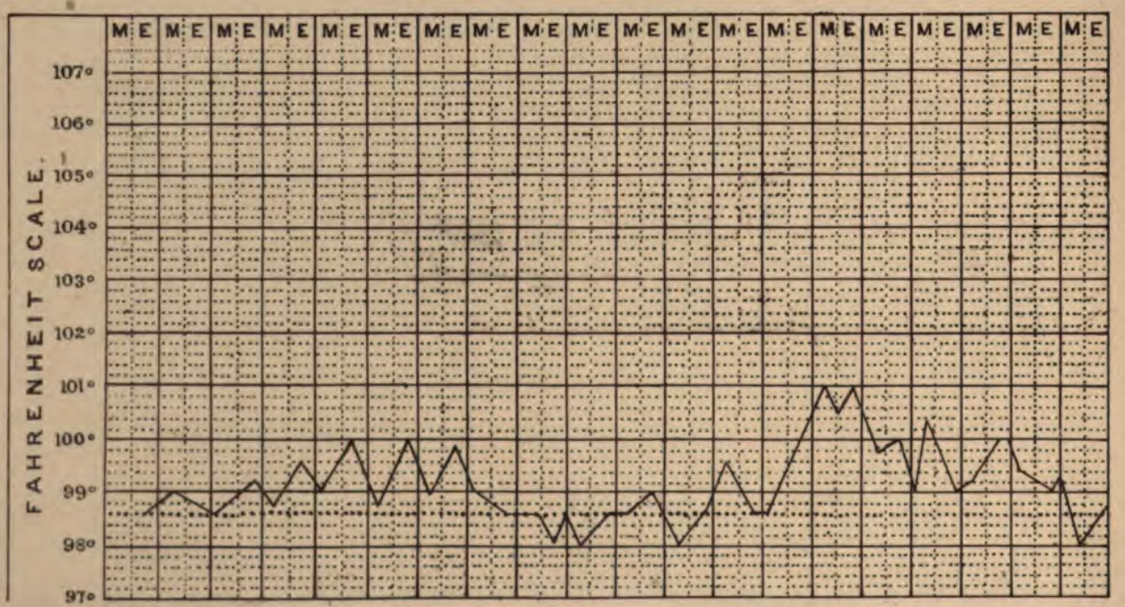
Septic conditions associated with the formation of infarcts in the organs are sometimes superadded.

The small intestine rarely shews any lesions. The heart, kidneys, brain, gall-bladder, urinary bladder, pancreas, suprarenals, testicles, lymphatic glands and muscles shew, as a rule, no macroscopic changes.

The parasites are found constantly in immense numbers in the cells of the liver, spleen and bone-marrow, in granulation tissue in the intestine and in the skin. They were present in large numbers in a lymphatic gland draining an area in which a skin lesion was present, but not in glands where no such condition existed. Bodies are, in some cases at least, fairly numerous in patches in the testicle. In the kidneys they do not appear to be numerous. They are present in some



CASE 1.—Advanced case of the disease. Spleen reaching to umbilicus. Papules and small ulcers. Diarrhœa.



CASE 2.—Well developed disease. Spleen well below the costal margin.

cases in the lungs. In the blood of the large veins they appear to be fairly numerous included in various types of cells.

The Clinical and Pathological nature of the disease.

In the first report I drew attention to certain very constant clinical features in cases of systemic infection by the parasite. These were—

(1) A great enlargement of the spleen.
(2) Emaciation.
(3) An irregular high temperature.
(4) Abdominal symptoms.

I have since been able to add the following which appear to be equally characteristic.

(5) A tendency to *noma* and local gangrenes.
(6) The frequent presence of a papular eruption and small or large ulcers of the skin.
(7) The occurrence of hæmorrhages.

Enlarged spleen.—Enlargement of this organ has been very constant. In Madras, bodies are almost always found on puncture in spleens which are so enlarged as to project several inches below the costal margin. On the other hand, in a few cases the spleen has been only moderately enlarged. In two cases there was a high temperature and severe constitutional disturbance associated with considerable enlargement of the spleen. Bodies were found, but were extremely scanty in both cases.

Emaciation.—Although emaciation is generally present, it is not invariably so, and death may occur from several causes connected with the disease without wasting being a prominent sign.

Temperature.—An irregular temperature has been noted as a feature of the disease. This is almost always present in the final stages. But even when splenic enlargement is considerable and emaciation marked, a high temperature may be absent, or slight rises only above the normal may occur. The most frequent type of temperature is that shewing irregular rises to 102° or 103° F.; at times a temperature of 104° or even 105° is reached. The two charts which accompany this report, for which I am indebted to Major Robertson, I.M.S., are typical of the disease as most frequently seen. There appears to be an acute form of the disease. In two cases in which the spleen reached half way to the umbilicus there was high fever which afterwards fell to normal as in enteric fever. In both cases it was very noticeable that the bodies were present in very small numbers in blood drawn from the spleen. Major Robertson, I.M.S., informs me that such cases are frequently readmitted to hospital with similar attacks.

Abdominal symptoms.—The constancy with which abdominal symptoms supervene has been already commented upon. These were shewn in the first report to depend upon the formation of extensive ulcers in the large gut. Of the seven autopsies, in five there was ulceration of the large intestine, and in one gangrene of the cæcum. In one case only was the mucous membrane of the large intestine normal. The dangerous nature of intestinal conditions is well shewn in the autopsies recorded, where three out of seven deaths were directly due to perforation of the gut. Healing of the ulcers appears to take place, since in one case, only numerous pigmented and depressed scars were present.

In the first report it was shown that the above lesions were associated with the presence of the parasites, often in very large numbers. In autopsy 6, single bodies were found scattered through the organised granulation tissue, and in the young tissue about the crypts, large cells were found as in autopsy 3. Bodies which appeared to be multiple division forms appeared more common in the granulation tissue from the intestine of case 3 than in the spleen, but the rapid changes in the tissues of the intestine leading to fragmentation of the nuclei render it difficult to be sure of such forms.

The amœba coli is by no means uncommon and is found in the granulation tissue in the intestines and in the walls of abcesses in the liver. How far the intestinal condition is due to the presence of this organism it is impossible at present to say. Amœbæ were found in cases 2, 3 and 5. Small amœbæ with a single chromatin mass were seen in the intestine as well as large forms in which the chromatin mass has undergone division. In the liver abscesses large circular forms were seen. The intestinal forms were identical with those seen in a typical case of severe dysentery in a European. I have not been able after long search to find any forms transitional between the amœba and the parasites under discussion. In two deaths from typical dysentery in which the amœba coli was present, the new parasite was not found in the spleen.

The occurrence of noma and local gangrenes.—Of the seven cases recorded, two died of *cancrum oris*, one of *noma* of the vulva, and one of gangrene of the cæcum. In two of these cases emaciation was not present and the body appeared well nourished. In one case there was, in addition to the *noma*, a large ulcer of phagedenic type which had exposed the bones of the foot.

Noma undoubtedly plays a most important part in bringing about death from this disease. *Noma* and perforation of the large intestine would appear indeed from our cases to be the chief causes of death in infection by the parasite.

Skin eruption and the occurrence of ulcers: Ulcers.—Shortly after the discovery by Wright of the bodies in tropical ulcer, Major Donovan,[6] reported the presence of the parasite in small ulcers which are often found about the knees

and elbows of cases of the disease. I have examined scrapings and small pieces of tissue snipped from such ulcers in three advanced cases of the disease and have found bodies in small numbers in each case. The ulcers usually seen are small, from 2 to 10 *m.m.* in diameter. They are covered with a thick raised scab and the base of the ulcer is usually depressed and devoid of granulations. The edges are usually thickened. The origin of the ulcers is doubtful. I have always failed to detect sarcoptes in them and they appear to arise from ulceration of a papular eruption which is generally found along with them.

In some cases larger ulcers are seen about the legs. They vary from the size of a shilling to extensive raw surfaces several inches in diameter. In autopsy 7, ulcers of this nature in section shewed scattered bodies throughout the tissues of their bases, and in the healthy skin in the immediate neighbourhood Very scanty bodies were also seen in snippings taken from such ulcers in a similar case (Pl. II, fig. 12).

In the granulation tissue surrounding the caseous mass in the dermis of case 5, bodies were present in considerable numbers, and large numbers of bodies were found in sections of a femoral gland above this lesion.

I have examined a number of large and small ulcers in the out-patient department, but so far have never detected bodies where there was no general infection with the new parasite.

Papular eruption.—It is common in cases of the disease especially in the advanced stages to find a scanty or profuse papular eruption about the thighs, Scarpa's triangle, and the scrotum. Similar papules are less frequently seen on the trunk, arms and neck. Some of these appear to ulcerate and form small ulcers covered with a raised scab, which are evidently slow to heal and chronic in nature. An unulcerated papule from a case shewed, in section, bodies in small numbers scattered through the dermis. Since we have been unable to find bodies in normal skin, it is possible that the eruption is an integral part of the disease. It appears distinct from the scabies seen on the hands of many of the cases, and the papule examined shewed a deeper lesion than in this condition.

With regard to the skin lesions in general I am unable to say whether they are due to the disease or other causes. The lesions have no very marked characters by which they may be detected or differentiated from those occurring in natives not suffering from the disease. On the other hand, the presence of the parasites in them is important.

Hæmorrhages.—Small petechial hæmorrhages are of fairly constant occurrence in fatal cases. They are especially frequent in the serous membranes, peritoneum, pleura and meninges. Larger extravasations of blood in similar positions and into the mucous membranes appear also to be not infrequent. In one case death occurred from a large hæmorrhage under the dura mater.

The relation of the Parasite to the tissues of the Host.

SKIN.—1. An unulcerated papule was removed from the thigh of an advanced case of the disease. The tissue was fixed in absolute alcohol, embedded in paraffin and thin sections stained by the modification of Romanowski noted in the first report.* Examination shewed in the deeper parts of the corium a layer of small round celled infiltration which refused to take on the Romanowski stain and appeared to be necrotic. Scattered throughout the dermis were isolated parasitic bodies. There were rarely more than three or four to be seen in any one field of the microscope. They occurred close beneath the epithelium in the dermal papillæ and to the full depth of the tissue removed (about 2 *m.m.*). At first sight the bodies appeared to have no relation to the blood vessels and to be situated in tissue cells. Close examination shewed that such bodies were in every case in the cytoplasm of cells which appeared to be endothelial in nature and which I believe to be the endothelium of very fine dermal capillaries. (Pl. I. fig. 4, and Pl. II. fig. 13.) In most cases the bodies were not surrounded by a vacuole. Occasionally a distinct vacuole was present.

2. Thin slices of ulcer margins from autopsy 7 were fixed in absolute alcohol embedded in paraffin and sections treated with Romanowski's stain. The base of the ulcers to the depth of 2·3 *m.m.* was formed of vascular tissue largely composed of young fibrous tissue cells. Single parasites were seen in the endothelium of

* NOTE.—The method referred to is as follows.

Small slabs of tissue 1 to 2 *m.m.* thick are placed on cover glasses to retain their shape and left in absolute alcohol until complete dehydration has taken place. If necessary, the alcohol should be treated with anhydrous copper sulphate freshly prepared and several changes of the alcohol should be made. Embed in paraffin, using xylol (15 minutes) to displace the alcohol. Leave in melted hard paraffin, 20-30 minutes. Sections are cut as thin as possible and flattened by floating on water just hot enough not to melt the paraffin. Float sections on to a slide, remove excess of water by tapping the end of the slide and press firmly over the sections with a piece of clean filter paper. No fixative is used. Dry above the flame taking care not to melt the paraffin. When quite dry, melt the paraffin and treat as usual with xylol, absolute alcohol and distilled water. Stain for 10 minutes in 1-1,000 eosin (höchst B. A. Grubler).

Pour off the excess of eosin, rapidly pour over the section some distilled water, cover with an excess of methylene blue solution prepared according to the following formula, and allow to stain for 15-20 minutes. It is a good plan on first adding the mythylene blue stain to keep the stain moving by tilting the slide as this tends to prevent deposit.

100 c.c. of 1 per cent. methylene blue (pure medicinal, Grubler) solution in distilled water.

5 c.c. of a 10 per cent. solution of chemically pure sodium carbonate.

Leave in the tropical sun until a strong red colour is seen on shaking.

Dilute 25 times for use.

Pour off excess of the methylene blue and wash momentarily in 70 per cent. alcohol. After the alcohol, plunge as rapidly as possible into distilled water. If there is still any precipitate left, repeat the process, until the section appears quite clean under a low power.

Wash in 1 to 400 acetic acid in distilled water, and place at once after washing in distilled water. Repeat the acetic acid bath until the tissue becomes free from blue stain and the nuclei are a clear bright red.

Allow the section to dry upon the slide. Mount in Canada balsam or allow to remain uncovered as in the case of a film. Sections mounted in Canada balsam last two or three months but eventually lose their colour.

The method is applicable also to tissues such as areolar tissue or muscle which can be spread upon a slide by the half-dry method.

fine capillaries throughout the whole area of the section including the dermis beneath the unulcerated epidermis. In the vessels above the size of capillaries, large cells containing from 6 to 12 bodies were occasionally seen.

3. Thin slices of the caseous nodule and the surrounding tissue described in autopsy 5 were fixed in absolute alcohol, cut in paraffin, and stained with Romanowski's stain. The central caseous mass was seen to consist of necrotic cells of several varieties, chiefly small lymphoid cells and large mononuclear elements. A certain number of polymorphonuclear cells were present, but they did not form the great majority of cells as in ordinary pus. The necrotic area was surrounded by granulation tissue which extended to a distance of a few millimetres between the layers of dense fibrous tissue, which surrounded the whole. Bodies were found readily in the granulation tissue, and wherever an infiltration of healthy tissues with cells was taking place. As many as ten, and in some cases more, bodies were to be seen in each field of the microscope. In the young granulation tissue composed of lymphoid cells and larger mononuclear cells the parasites were seen in every case lying embedded singly in the latter type of cell. In the capillaries in the neighbourhood, bodies were seen lying singly, or in twos and threes, in the endothelium cells. In the smaller vessels in the neighbourhood of the granulation tissue, large cells containing numerous parasites were seen. These were similar to the cells seen in the liver and spleen. They were most often attached by one side to the vessel wall whilst the mass of the cell projected into the lumen of the vessel (Pl. II. fig. 9). In the somewhat larger vessels they were often much more free in the lumen. In a sweat gland which had been invaded by the granulation tissue, many cells, each containing several bodies, were seen. These lay in close connection with the coils of the gland, apparently in fine capillaries which surrounded the coil. In adipose tissue which had been invaded by granulation tissue, single bodies lying in the endothelium cells of capillaries were readily seen. In parts of the section remote from the granulation tissue, bodies could not be found either in the capillaries or larger vessels. In the capillaries of healthy adipose tissue no bodies were to be made out. In these sections all stages between unaltered endothelium cells containing a single body, and large cells partially detached from the vessel wall and containing many bodies, could be made out.

4. Sections of apparently normal skin from the thigh of the same case were carefully searched, but no bodies were found. In the neighbourhood of the pale cicatrix-like areas already noted, many large cells loaded with pigment of the same nature as the epithelial pigment were seen in the tissues and occasionally in the smaller vessels.

5. Areolar tissue from the skin and subperitoneal tissue were spread upon slides and stained by the Romanowski method. The tissue elements were well displayed, but no bodies were seen.

6. Films from various sized chronic ulcers apart from general infection have failed to shew parasites. In ulcers encroaching upon the skin, large cells with single or double nuclei, and exactly resembling the smaller macrophages, are seen filled with balls of a greenish pigment which appear to be derived from the skin.

LYMPHATIC GLANDS.—A femoral lymphatic gland taken from the right groin in case 5, cut in paraffin and stained by Romanowski's method shewed, in the lymph sinuses lying around the cortical masses of lymphoid tissue, many large cells crowded with the parasitic bodies. Similar cells containing pigment of the same nature as that seen in sections of the skin were also present in the same position, and in some cases cells contained both pigment and parasites (Pl. I. fig. 6). Many of the cells shewed the necrotic changes and central vacuolisation to be described later (Pl. II. fig. 6). In some cases they appeared to be on the point of dissolution. Many of the stroma cells of both the cortex and medulla, but especially the former, contained small groups of the bodies (Pl. I. fig. 5). Sections of a femoral lymphatic gland from case 6, in which no skin lesion was present, shewed no bodies.

INTESTINE.—Very thin sections of the intestine in autopsies 3 and 6 shewed single bodies lying in the organised granulation tissue. In almost every case they could be demonstrated as lying in what appeared to be the cytoplasm of endothelium cells of fine capillaries. In the younger granulation tissue, and especially that infiltrating between the crypts of Lieberkuhn, the bodies were seen in large cells resembling stroma cells, and in macrophages. The number of bodies in different pieces of tissue from the same case varied very much.

TESTIS.—Sections of the testis from autopsy 5 shewed among the tubules small areas in which an aggregation of nuclei was apparent. On closer examination these nuclei were seen to be chiefly those of endothelium cells. In these areas single bodies were present in considerable numbers lying in the cytoplasm of the cells. In somewhat larger vessels, endothelium cells were seen containing two or three bodies (Pl. I. fig. 3) and in the still larger vessels crowded cells were frequent. The bodies were not present in the capillaries in all parts of the testis.

LUNG.—In autopsies 4 and 5, fairly numerous large crowded cells were seen in films.

KIDNEY.—Films from autopsy 6 shewed occasional cells of endothelial nature containing from 5 to 20 bodies. Only a small piece of kidney cortex was reserved for sections and no bodies could be seen in this. The glomerular capillaries were carefully searched but in vain. Sections of the cortex and medulla of the kidney from case 1 were examined but no bodies were found.

LARGE VEINS.—Blood films made from the large veins, femoral, portal, hepatic, some hours after death, shewed cells containing parasites which were first thought to be peculiar forms of large mononuclear leucocytes. It seems certain that

these cells are in reality endothelial in nature, since they resemble closely cells seen forming the walls of small vessels exposed in tangential section. I have thought it possible that they may become detached from the vessel wall after death (Pl. II. fig. 6 and fig. 7).

SPLEEN AND LIVER.—In blood obtained from these organs by puncture, most of the parasites are seen either free or included in a matrix. In sections of the organs no such relation is to be made out and the parasites are seen lying in the cytoplasm of cells. The cells are of two kinds.

Leucocytes.—Laveran and Mesnil, Ross and Manson and Low all call attention to the existence of phagocytosis and the presence of bodies in the large mononuclear and polymorphonuclear leucocytes. In my first report I have also considered the part played by the leucocytes in taking up the parasites.

The endothelium.—Marchand and Ledingham note the occurrence of the parasite in very large cells in the spleen and bone-marrow. In my first report I also made mention of these enormous cells, which appear to be of a similar nature to the macrophages seen in fatal cases of malaria. The careful study of sections of the spleen and liver in cases of infection with the new parasite has led me to conclude that the macrophages are derived from the vascular endothelium and that they represent a final stage of endothelium cells which have become more and more modified and distended with included parasites. It is indeed easy to trace every gradation between flattened endothelium cells containing a few bodies and the enormous swollen cells almost blocking the capillary in which they lie. In both the liver and spleen, many more bodies are included in cells of endothelial nature than in the leucocytes. In films this fact is masked by the rupture of many of the more distended endothelial cells, and also by the fact that many endothelium cells approximate in appearance to cells of large mononuclear leucocyte type and may be readily classed in this group. Two cells, in reality endothelial in nature, are figured by me as large mononuclear leucocytes in my first report (fig. 22) and it is possible that others have erred similarly. The following types of endothelium cell in which the parasite occurs may be noted.

1. Endothelium cells but little modified. In films these have round, oval or kidney-shaped nuclei and extensive protoplasm which is often arranged so as to give the cells an elongated appearance. The protoplasm, as a rule, shews a tendency to vacuolisation especially towards the free ends of the cells (Pl. II. fig. 6). It also tends to become protruded in the form of buds. In sections they may be quite flat or they may shew at one, or more rarely both ends, a swollen appearance. They are applied closely to the capillary wall or stroma. They contain, as a rule, from 6 to 12 bodies. Identical cells are seen in the capillaries of the testis and of granulation tissne (Pl. II. fig. 8).

2. Large rounded cells with extensive protoplasm and a round or oval nucleus. Small specimens of these cells may give rise to a puzzling appearance in films since they resemble, as far as the nucleus goes, a small mononuclear leucocyte. The protoplasm is, however, much more extensive than is the case with the leucocyte. Even large cells of this type are often seen whole or only partially disrupted in films. Their protoplasm is often like ground-glass in appearance and is usually vacuolated. Masses of the protoplasm of these cells readily become separated from the cell (Pl. II. fig. 4), and may assume a globular shape. They contain often 20 or more bodies. In sections of the testis and of granulation tissue, cells which appear to be of this type are seen in the smaller vessels attached as a rule by one side and projecting freely into the lumen. Similar cells are seen in blood taken *post-mortem* from the large veins, femoral, portal, hepatic. (Pl. II. figs. 7 and 9.)

3. In both the liver and spleen, immense numbers of very large cells are seen lying in the capillaries. They may be extended along the capillary, or their cytoplasm may be massed together. In the spleen, large cells are seen with long processes extending in among the smaller cells of the pulp. Both types of cell appear to be of the same nature. They have a single or double vesicular nucleus, and their cytoplasm, which stains lightly, has numerous included bodies lying either scattered more or less evenly throughout, or in groups.

4. In both the liver and spleen, but especially in the latter, extremely large cells are seen in which the nucleus is small, stains intensely and is pushed to one side or extremity of the cell. The cytoplasm of these cells stains more darkly than does that of the last mentioned variety, and in some cases takes on a curious dirty hue suggesting marked necrotic changes. The centre of the cell is occupied by a large vacuolated space, and arranged around this are numerous parasitic bodies. The cytoplasm of many of these cells is reduced to a mere pellicle containing a crowd of parasites, and such cells appear to be on the point of rupture. Cells of this and the last mentioned type are rarely seen whole in films unless special precautions are taken by fixing in $\frac{1}{4}$ per cent. osmic acid. One such cell in the bone-marrow contained as many as 250 bodies (Pl. II. figs. 10 and 11), also (Pl. I. fig. 6).

Peripheral blood.—Laveran and Mesnil[3] record the presence of bodies in the peripheral blood which they take to be endoglobular forms of the parasite. Such forms, they say, are very rare, smaller than those found in the spleen and have only one chromatin mass. I have been unable to satisfy myself that undoubted bodies occur in the peripheral blood, free or in the red cell. Taking into account the negative results of Manson and Low who examined a case with this object very systematically and my own results on a number of cases, it does not appear that typical forms are present. Laveran and Mesnil's forms do not appear

to me very convincing, and even these must be of extreme rarity. In two advanced cases approaching a fatal termination, I have found in the peripheral blood a considerable number of typical forms possessing double chromatin masses and in every way resembling those found in the spleen, but these were all included either in polymorphonuclear or mononuclear leucocytes. In one case 37 parasites, nearly all in polymorphonuclear leucocytes, were seen during a count of 500 leucocytes. In the other case, 7 parasites were seen whilst counting a similar number of leucocytes. In both cases leucocytes were in excess of the number usually found in the disease in which, as a rule, the most marked leucopœnia is found. In neither case did I find at this time free forms or forms in the red cells. In several instances I have examined very carefully films of splenic blood with a view to finding transitional forms between the bodies lying in a matrix and those in unchanged cells, but unsuccessfully. I am led, then, to doubt very greatly the specific nature of the bodies in the red cells.

In the most severe infections the relative leucocyte values do not appear to shew very great changes. The leucopœnia is the most marked change, and this is, as a rule, so great that it is necessary to take several large films in order to make adequate leucocyte counts. Examples of the values obtained are the following.

CASE 1.—Total leucocytes counted, 500.

Polymorphonuclear leucocytes	60 per cent.
Large mononuclear ,,	11·4 ,,
Small ,, ,,	24 ,,
Eosinophyl cells	1 ,,
Myelocytes	1·2 ,,
Transitional	1·8 ,,
Intermediate	·6 ,,

CASE 2.—Total leucocytes counted, 500.

Polymorphonuclear leucocytes	69·8 per cent.
Large mononuclear ,,	8·6 ,,
Small ,, ,,	20·4 ,,
Eosinophyl cells	·4 ,,
Myelocytes	·2 ,,
Transitional	·6 ,,
Intermediate	0·0 ,,

CASE 3.—Leucocytes 250 per c. m. m. Total number counted 300.

Polymorphonuclear leucocytes	56 per cent.
Large mononuclear ,,	10·6 ,,
Small ,, ,,	29·2 ,,
Eosinophyl cells	3·6 ,,
Myelocytes	·6 ,,

CASE 4.—Leucocytes 437 per c. m. m. Total number counted 500.

Polymorphonuclear leucocytes	73 per cent.
Large mononuclear ,,	7 ,,
Small ,, ,,	16 ,,
Eosinophyl cells	4 ,,

THE NATURE OF THE INFECTIVE PROCESS.—I have shewn that in whatever position the bodies are found they are almost invariably contained in the cytoplasm of cells. The large numbers of free forms seen in films of blood obtained by splenic puncture can only be considered as forms artificially set free from the cells they normally occupy. Two types only of cells have been so far observed to harbour the parasite, *i.e.*, the leucocytes and cells of endothelial nature. It is above all the vascular endothelium which is involved in the type of infection giving rise to splenomegaly. That the vascular endothelium plays an important part in collecting the pigment of malaria is well known, but in the present case there is the additional and most important fact that inclusion in the cytoplasm of the cell is not prefaced or followed by the destruction of the parasite. We have in so called malarial cachexia a condition characterised by the presence of enormous numbers of the parasite in the viscera. At the same time the parasites are with the greatest rarity to be found outside the endothelium or leucocytic cytoplasm. From the inclusion of single bodies in the endothelium of certain capillaries to the rupture of the large necrotic cells in the spleen and elsewhere, the life of the parasite appears to be spent in the cytoplasm of an endothelium cell. On the dissolution of the necrotic cells it would certainly appear that the bodies are set free into the blood stream. Even then they must be again very rapidly taken up again by cells, since it is acknowledged by all observers that typical free forms in the peripheral circulation are excessively rare. The absence of free forms in the blood of two cases where the parasite was readily found in the leucocytes points to the same conclusion. It appears very probable that inclusion in the cells of the body is not prejudicial to the life of the parasite, and that it undergoes development by fission normally in the cells of its host. Whether the great increase in the number of bodies in the endothelial cells is mainly due to the phagocytic activity of the cell or to multiplication of the parasite within its substance one hesitates to decide. The reduction of many of the cells to mere bags containing multitudes of parasites certainly suggests that active multiplication of included forms takes place.

With regard to the part played by the spleen and liver it is as yet premature to form conclusions. On the whole it is probable that auto-infection of the splenic and hepatic endothelium is constantly going on and forms the chief process in the disease. The fact that bodies are found in the skin, intestine, lungs and testis as well as in the large veins and in the lymphatic glands, draining certain lesions, shews that wide dissemination of the parasite through the body also occurs, and the function of the spleen and liver as storing organs must not be overlooked.

It is of course possible that the endothelium infection is derived primarily from very rare forms living in the red cells, but it seems, in the present state of

our knowledge, much more probable that the final disintegration of large necrotic cells is the means by which fresh swarms of the bodies are set free to be taken up again by the endothelial cells of the spleen and elsewhere.

A consideration of the main features of the process here exemplified, namely, an endothelial infection, invites comparison with the processes in certain other diseases.

DISEASES OF SEPTICÆMIC TYPE.—Infection by the new parasite as shewn in so-called malarial cachexia has a strong resemblance to certain chronic septicæmias, notably to some forms of malignant endocarditis.

That destruction of red cells is not necessarily due to actual invasion of the corpuscle by the parasite is shewn in those cases of septicæmia where the destruction of red cells is usually very great.

The finding of organisms in the circulating blood of cases of septicæmia and pyæmia is also generally difficult and necessitates the use of considerable quantities of blood, a point which has been elucidated especially by Kuhnan.[4] The experiments of Werigo[5] and others indeed shew that it is the endothelium and leucocytes in the visceral capillaries which are mainly implicated when an infection of the blood stream by micro-organisms takes place. We may indeed almost consider this form of splenomegaly as a septicæmia in which the new parasite is the infecting organism. We do not yet know how the parasite enters the blood stream or whether it may give rise to conditions other than those found in splenomegaly or in tropical ulcer, but in these we appear to have two very distinct types of infection.

(1) A local lesion—Tropical ulcer.

(2) A septicæmia—the so-called malarial cachexia of India.

The fact that an infection may be mainly or entirely endothelial appears to me to be important. The examination of the peripheral blood in Indian cachexia is scarcely likely to have led to the discovery of the parasite. In Malta fever splenic puncture is generally necessary in order to detect the infecting micro-organism. Even in pronounced septicæmias, where cultural methods can be employed, the detection of forms in the blood is uncertain, and a negative result generally recognised as being without significance. By puncturing the spleen one removes many endothelial cells and leucocytes which have remained in the visceral capillaries, thus tapping an entirely different tissue from the blood. Such a means of investigation should be a fruitful one in diseases where a blood infection appears probable, but where no organism is to be detected in the peripheral blood, *e.g.*, yellow fever.

DISEASES OF GRANULOMATOUS TYPE.—Tropical ulcer has many features which would lead to its classification as a granulomatous lesion. Both Cunningham and Wright shew that extensive deposits of granulation tissue are

formed in the dermis quite apart from ulceration, which is evidently a secondary process. That the organism under discussion is the cause of this deposit cannot be doubted in the face of Wright's description. It is difficult to conceive of the massing of a parasite of the red cell in such a position, and we most probably have in tropical ulcer a granulomatous lesion in which the new parasite is the exciting cause. It is premature to discuss the relation of the systemic infection by the parasite to diseases of granulomatous type. Much depends upon the relation of the disease to the small ulcers and papules described in this paper.

The morphology of the parasite.

I have already drawn attention to the presence, noted by all observers, of two chromatin masses in the bodies. I shewed that the arrangement of these and the structure of the parasite in general is remarkably constant, and that a schema of the parasite could be devised which would exhibit when viewed from different directions most, if not all, the appearances shewn by the parasite in films. The "tail" described by some authors as joining the large and small nucleus I considered a strand or ridge of protoplasm left between two vacuoles. In a proportion of the bodies, especially in the larger forms, I have since made out a further detail of structure. This is a very distinct darkly-stained line, starting from the neighbourhood of the small chromatin mass and ending as a rule abruptly at a distance from the large mass. This line is nearly always at right angles to the tail previously described, and by means of the schema it is possible to make out that it lies in the line of division of the two hypothetical valves (Pl. II. fig. 1).

In the first report I described the appearance in the bodies of a distinct cuticle. Ross [7] draws attention to the same appearance and the bodies as a rule shew it to a marked degree. Instances of apparent rupture of this cuticle are often seen (Pl. II. fig. 2).

In some cases, especially when the tissues are taken *post-mortem*, the bodies appear as protoplasmic masses only, and the appearance of a cuticle is absent. The bodies also undoubtedly rapidly break up after the death of the host. There appears some doubt then as to the resistant power of the apparent capsule, and the appearance may be deceptive. As the bodies appeared not to be piroplasma, and as from their number, their size and their morphology they appeared to resemble somewhat the spores of microsporidia, I have made many attempts to cause protrusion of the filament but without success. Blood from the spleen containing the bodies was mixed with ether, strong and weak acids and ammonia. Films were then made, thoroughly washed first in alcohol and then in water, and stained by Romanowski's method. Films made from blood containing the bodies

were placed, without being allowed to dry, over the fumes of strong acids and the vapour of ether. In most cases the bodies were to be readily made out, and, even in the case of the strong acids, stained characteristically. No appearance of a filament was detected.

The matrix.—Laveran, Ross, Manson and Low all describe a substance in which many of the bodies are embedded. In films made from blood drawn from the spleen during life this "matrix" is much in evidence. In films made *post-mortem* it is not to be made out. The appearances are not quite the same in every case and the matrix may be seen in one or other of the following forms.

(1) As irregular, fragmented and ragged masses. It may be a small and very faint structure, often just visible, or it may be of considerable size. Large masses of this nature, many times larger than a blood corpuscle, may often be seen containing only a single body (Pl. II, figs. 3 and 4).

(2) As well-defined globular masses, smaller than, the same size as, or larger than, a red corpuscle. The substance is as a rule finely reticular. It may be like ground-glass in appearance. Identical bodies can be seen quite free from included parasites, and budding from large cells of endothelial nature (Pl. II, fig. 5).

(3) Occasionally a faintly staining very hyaline substance, small in amount, may be seen in which from 5 to 10 bodies are embedded. The bodies often lie in vacuoles slightly larger than themselves. The amount of matrix in this case is always very small, and it is never found projecting beyond the included bodies.

As regards the appearances described above, there can be no doubt that (1) and (2) are the fragmented and budded cytoplasm of cells of endothelial nature. The undoubted presence in some cases of pigment, either malarial or derived from the skin and identical with pigment seen in the cells in sections, definitely demonstrates their nature.

The third type, which is much rarer than the others, appears possibly to be residual matter left over from the separation of the multiple division forms described first by Laveran. In the first report I figured bodies having a clear circular outline and containing three or more large, and an equal number of small, chromatin masses, but in which no division into individual bodies could be made out. In sections of the spleen I have been able occasionally to see such forms. They contain as a rule about six large and six small masses. The large masses are arranged peripherally and the small masses centrally. Such forms are enclosed in the cytoplasm of cells as in the case of the bodies ordinarily seen.

Infection of other hosts than man.—I have not succeeded in producing infection by inoculation of the bodies into animals. Monkeys and rabbits were used, and blood containing the bodies was injected subcutaneously into the peritoneum and into the blood stream (Intra-ventricular injection). Up to three weeks after intravascular injection, and six weeks after injection into the tissues

and the peritoneum, no lesion had been produced and no bodies were found in the spleens of the animals inoculated.

Comparison of the conditions found *post-mortem* in infection by the new parasite, and in Trypanosome infection.

The organism used for comparison was a highly pathogenic trypanosome which occurs naturally in the pariah dogs of Madras. This trypanosome causes death in dogs in from 6 to 7 weeks after inoculation, and in rats in from 8 to 10 days. The symptoms caused were emaciation, weakness, slight œdema of the tissues about the eyes, a scurfy skin and distinct paresis of the hind legs. In advanced stages of the disease the trypanosomes were abundant in the peripheral blood.

In animals some hours dead, the trypanosomes were found in the large veins in masses nearly as large as the field of the microscope. They shewed marked changes. The general shape of the trypanosome was not in most cases recognisable. The large and small chromatin mass (nucleus and centrosome) were approximated, and in some cases shewed a remarkable resemblance to the chromatin masses of the new parasite seen *post-mortem*. Numbers of filaments were everywhere to be seen. They, however, did not stain very deeply and in some clumps were by no means conspicuous. Nothing like the definite outline of the new parasite was ever seen and the resemblance was evidently superficial only.

The spleen in the case of three dogs killed on the point of death was somewhat, though not greatly, enlarged. Though the trypanosomes were abundant in the peripheral blood, they were rare in the spleen both in the case of dogs and of rats. It was difficult to find forms included in the spleen cells. When found, such forms shewed two chromatin masses approximated so as to resemble those of the new parasite, and as a rule no filament was seen. In some cases both the filament and body were still to be recognised. In no case was the outline, so characteristic of the new parasite, seen. The large crowded cells, seen in infection with the latter, were absent. In none of the animals examined did there appear to be a storing of forms in the spleen. Sections of the spleen shewed occasional double chromatin masses in cell protoplasm, but the appearances were otherwise quite unlike those seen in infection with the new parasite. The storing of enormous numbers of the new parasite in an apparently unaltered state in the splenic cells is one of the most remarkable features of infection by these bodies. In trypanosome infection of the dog such a phenomenon does not appear to take place, and from the paucity of included forms one is led to conclude that phagocytosis is not great or that intracellular digestion is rapid.

In a dog strongly infected with *piroplasma canis* many of the leucocytes

contained included forms. Some of the forms shewed double chromatin masses and had, as in the case of included trypanosomes, a slight resemblance to the new parasite. In spite of many examinations nothing more than this superficial resemblance was encountered. Contrary to the state of affairs in the case of the new parasite, intracellular digestion appeared to be in progress and many forms shewed disorganised and scattered chromatin.

Conclusions.

1. The bodies described by Wright in tropical ulcer are indistinguishable from those found in cases of enlarged spleen in Madras. In both cases the bodies are for the most part included in the cytoplasm of cells of endothelial nature. In tropical ulcer the bodies appear to be giving rise to a lesion of a distinctly granulomatous type.

2. In so-called malarial cachexia of India the bodies are very numerous in the spleen, liver and bone-marrow. They are in considerable numbers, in some cases at least, in the lungs and testes. In the kidney they do not appear to be in large numbers. In the above named viscera they may occur in leucocytes, but for the most part they are seen in cells of endothelial nature, especially in large cells crowded with the bodies (macrophages).

3. Bodies may be present in large numbers in the granulation tissue associated with ulceration of the large intestine in cases of so-called malarial cachexia.

4. Bodies may generally be found in small numbers in the granulation tissue of small and larger ulcers, and in unulcerated papules in the skin of advanced cases of so-called malarial cachexia. In this case, as in the testis, the bodies are found for the most part lying singly in endothelial cells of the finest capillaries.

5. Bodies were found in a lymphatic gland which drained a skin lesion containing the bodies. They were not found in a lymphatic gland draining only normal skin.

6. Bodies may be found in leucocytes in the peripheral blood. I have not seen unmistakeable forms in red cells either in peripheral or splenic blood.

7. The vast majority of the bodies lie in the cytoplasm of endothelium cells. The cachexia is essentially an infection of the vascular endothelium and resembles in many ways a chronic septicæmia.

8. The process of infection appears to be as follows. Bodies are taken up by, or invade, endothelium cells in the visceral and certain other capillaries, *e.g.*, granulation tissue. The endothelium cells increase in size and become more and more distended with the parasites (macrophages). The cells finally undergo necrosis and appear as mere bags filled with large numbers of the parasites. Eventually such cells would appear to rupture and the contained bodies, which exhibit no trace of intracellular digestion, are thus set free to be taken up again by cells.

Explanation of Plates.

PLATE I.

FIG. 1.—Adipose tissue infiltrated by granulation tissue. The figure shews several bodies lying in the cytoplasm of cells. The section is from the edge of an ulcer. Case 5.

FIG. 2.—Young granulation tissue from the same ulcer.

FIG. 3.—Bodies lying in endothelium of a small vessel in the testis.

FIG. 4.—Body lying in cytoplasm of cell in the dermis. The section is from an ulcerated papule.

FIG. 5.—Large cells crowded with the new parasite in the lymphatics of a lymphatic gland. Case 5. Bodies are seen also in the stroma cells.

FIG. 6.—Large necrotic cell in same gland.

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Fig 1.

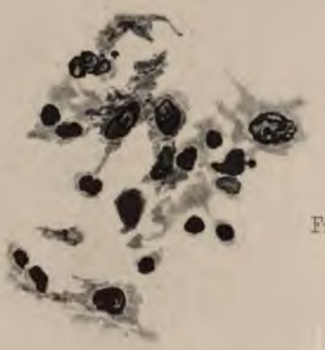

Fig 2.

Fig 3.

Fig 4.

Fig 5.

Fig 6.

Photogravure.

Survey of India Offices, Calcutta, May 1904.

PLATE II.

FIG. 1.—Bodies shewing curious tail-like structure, distinct from the "tail" often seen joining the two chromatin masses.

FIG. 2.—Bodies shewing appearance of a ruptured capsule.

FIG. 3.—Matrix. Faintly stained irregular mass.

FIG. 4.—Matrix. Cytoplasm of endothelium cell containing bodies. Unruptured endothelium cells are seen in films of splenic blood shewing an exactly similar cytoplasm. See also fig. 6 and fig. 7.

FIG. 5.—Matrix. Type 2. Shews mass much smaller than a red cell. Similar globular masses are seen, the same size or larger than red cells.

FIG. 6.—Endothelium cells from femoral vein.

FIG. 7.—Endothelium cell from femoral vein. Exactly similar cells are seen in film of splenic blood.

FIG. 8.—Section of endothelium cell slightly swollen and containing two bodies (Testis).

FIG. 9.—Section of endothelium cell much swollen and containing many bodies (Small vessel in granulation tissue).

FIG. 10.—Large necrotic macrophage (spleen).

FIG. 11.—Similar cell but reduced to a mere pellicle.

FIG. 12.—Medium sized and large ulcer on leg. Case of Splenic infection. Bodies were present in the granulation tissue.

FIG. 13.—Single body lying in cytoplasm of endothelioid cell. (Dermis. Unulcerated papule.)

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Fig. 9.

Fig. 10.

Fig. 11.

Fig. 12.

Fig. 13.

Photogravure.

Survey of India Offices. Calcutta. May 1904.

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BY

LIEUT. S. R. CHRISTOPHERS, M.B., I.M.S.

(*On special duty.*)

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